CONSOLIDATED REPORT ON MONSANTO'S SOYBEAN MON87705 APPLICATION FOR DIRECT USE AS FOOD AND FEED OR FOR PROCESSING (FFP)

EXECUTIVE SUMMARY

On December 18, 2018, Monsanto Philippines submitted soybean MON87705 application for direct use under the DOST-DA-DENR-DOH-DILG Joint Department Circular (JDC) No. 1 Series of 2016.

After reviewing the Risk Assessment Report and attachments submitted by the applicant, the STRP, BAI, and BPI-PPSSD found scientific evidence that soybean MON87705 is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health.

The Department of Environment and Natural Resources – Biosafety Committee (DENR-BC), after a thorough scientific review and evaluation of the accomplished Project Description Report (PDR) and Environmental Risk Assessment (ERA) form along with the submitted sworn statement and accountability of the proponent, reported that the direct use of the regulated article will not cause any adverse effect on the environment (land and water) and biodiversity.

The DOH-BC, after a thorough scientific review and evaluation of documents related to Environmental Health Impact, found scientific evidence that the GM application will not cause significant adverse effects to human and animal health, is unlikely to result in allergenic reaction, and is as safe as food or feed derived from conventional varieties.

Furthermore, the Socio-economic, Ethical and Cultural (SEC) expert, after reviewing thoroughly the accomplished SEC questionnaire, also recommended for the issuance of biosafety permit.

BACKGROUND

In accordance with Article VII. Section 20 of the JDC, no regulated article, whether imported or developed domestically, shall be permitted for direct use as food and feed, or for processing, unless: (1) the Biosafety Permit for Direct Use has been issued by the BPI; (2) in the case of imported regulated article, the regulated article has been authorized for commercial distribution as food and feed in the country of origin; and (3) regardless of the intended use, the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart.

The BPI Biotech Office provided the assessors the complete dossier submitted by Monsanto Philippines. Upon receipt of the individual reports from the assessors, the BPI Biotech Secretariat prepared this consolidated risk assessment report for the information of the public.

STRP, BPI-PPSSD, BAI (Safety Assessment)

1. **Host Organism** *(Glycine max (L.) Merr)*

Soybean has been extensively used as food and feed as a major source of protein as well as a major source of vegetable oil around the globe. It contains anti-nutritional factors such as oligosaccharides, trypsin inhibitors, lectins, phytic acid and other compounds such as isoflavones, phospholipids, sterols and saponins (OECD, 2012, Section II. pp. 30-35).

Soybean is one of eight allergenic foods that, in total, are responsible for approximately 90% of all food allergies. Soybean is less allergenic than other foods in this group and rarely responsible for severe, life-threatening reactions. Allergy to soybean is more prevalent in children than adults and is considered a transient allergy of infancy/childhood.

Consumption of soybean and products is at 18g/day in the Philippines, in the form of soy sauce at 15g/day, soybean milk-nil, and other soybean products at 3g/day. Soybean meal is the premier supplemental protein source in livestock and poultry rations due to its nutrient composition, availability, and price. Typically, soybean meal is used to meet the animal's requirement for limiting amino acids, as it is the most cost-effective source of amino acids.

2. Transgenic Plant

MON 87705 has been reviewed and approved for food and/or feed use in many countries including Australia/ New Zealand (Food, 2011), Canada (Feed, Environment, 2011; Food, 2011), China (Food, Feed, 2017), Colombia (Feed, 2012; Food, 2014), EU (Food, Feed, 2015), Indonesia (Food, 2013), Japan (Food, 2012; Feed, 2013; Environment, 2013), Korea (Food, 2013; Feed, 2012), Mexico (Food, Feed, 2011), Philippines (Food, Feed, 2014), Singapore (Food, Feed, 2015), Taiwan (Food, 2018*; Feed, 2018), United States (Food, Feed, 2011; Environment, 2011), Vietnam (Food, Feed, 2015).

Consumption pattern is not expected to increase upon introduction of the novel food because the modification of MON 87705 does not change the composition, safety or nutrition compared to traditional soybean except for the improvement in the fatty acid composition and glyphosate tolerance.

3. **Donor Organism**

MON 87705 insert contains a *cp4 epsps* expression cassette and a *FAD2-1A/ FATB1-A* suppression cassette.

The FAD2-1A/ FATB1-A suppression cassette encodes for double stranded RNAs (dsRNAs) which are found commonly in eukaryotes, including plants, for endogenous gene suppression and are composed of nucleic acids. Nucleic acids have a long history of safe consumption and are considered generally recognized as safe (GRAS) by the U.S. FDA. There is no evidence to suggest dietary consumption of RNA is associated with mammalian toxicity or allergenicity, and it is extremely unlikely to encode for a protein. Therefore, discussion relating to source and potential pathogenic or allergenic properties of the FAD2-1A/ FATB1-A sequence is not necessary.

The *cp4 epsps* gene is derived from the common soil bacterium, *Agrobacterium* sp. strain CP4. The donor organism, *Agrobacterium* sp. strain CP4, was isolated based on its tolerance to glyphosate brought about by the production of a naturally glyphosate tolerant EPSPS protein. *Agrobacterium* species are not known for human or animal pathogenicity, and are

not commonly allergenic. In addition, there is no known population of individuals sensitized to bacterial proteins. The history of safe use of *Agrobacterium* sp. strain CP4 has been previously reviewed as a part of the safety assessment of this donor organism for other Roundup Ready® crops.

4. Transformation System

MON 87705 was produced by *Agrobacterium*-mediated transformation of soybean with the binary vector PV-GMPQ/HT4404 that contains two transfer DNAs (T-DNAs). T-DNA I and T-DNA II both contain DNA segments designed to suppress endogenous *FAD2* and *FATB* genes which encode two key enzymes in the soybean fatty acid biosynthetic pathway. T-DNA I contains the sense segments of the *FAD2-1A* intron and *FATB1-A* 5' untranslated region (UTR) and plastid targeting sequence which are under the regulation of the *7Sα'* seed promoter. The T-DNA II contains a partial[5] suppression cassette that contains the antisense segments of the *FAD2-1A* intron and *FATB1-A* 5' untranslated region (UTR) and plastid targeting sequence. T-DNA I also contains a *cp4 epsps* expression cassette under the regulation of a promoter (*FMV/Tsf1*) active in all tissues. During plant transformation, the two T-DNAs (T-DNA I and T-DNA II) co-integrated into one locus in the soybean genome. The cointegration of the T-DNAs in this configuration creates an insert containing a single *cp4 epsps* expression cassette and a single *FAD2-1A/ FATB1-A* suppression cassette.

5. Inserted DNA

A single copy of the T-DNA 1 and T-DNA II was integrated into the soybean genome at a single integration locus. This was demonstrated by Southern blots analysis after digestion of MON87705 with restriction enzymes *Nco* I or *Spe* I.

The integrity and order of the genetic elements were demonstrated by PCR and DNA sequence analysis and the developer stated the genomic DNA extracted from MON 87705 shows that the DNA sequence flanking the ends of the insert is also found in the native soybean genome.

The insert in MON 87705 is 7251 base pairs and matches the sequence of PV-GMPQ/HT4404 as described. The sequence analysis showed a 30 bp truncation at the 3' end of the FATB1-A antisense segment, which did not have an impact on the ability of the suppression cassette to reduce levels of FATB1-A RNA and result in the intended fatty acid phenotype.

Sequence comparison between the PCR product generated from the conventional soybean (A3525) and the sequence generated from the 5' and 3' flanking sequences of MON 87705 indicates there was a 36 bp deletion (bases 896-931) and a 2374 bp insertion just 5' to the MON 87705 insertion site.

This analysis confirms that the genomic sequences flanking the insert in MON 87705 are native to the soybean genome and that a 36 base-pair deletion and a 2374 base pair duplication that contains a single base change occurred at the insertion site during integration of the T-DNA sequences. These molecular rearrangements presumably resulted from double-stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998).

6. Genetic Stability

The multigenerational stability of the introduced traits was assessed through Southern blot analysis of the genomic DNA from four generations. Results of the analysis showed that the inserted T-DNA in MON 87705 is stably inherited from one generation to the other (Skipwith A. et al., 2009).

Segregation analyses show heritability and stability of the insert occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the DNA insert at a single chromosomal locus.

The genotypic stability and segregation of T-DNA I and II in MON 87705 was confirmed by performing a Chi-square (χ^2) analysis on zygosity data generated for the *H6* 3'UTR genetic element over four generations. A Chi-square analysis was used to compare the observed segregation ratios to the expected ratios according to Mendelian principles.

The result of the genotypic stability and segregation of T-DNA I and T-DNA II in MON 87705 are consistent with molecular characterization data indicating a single genomic insertion site in MON 87705 and show the insert follows the expected Mendelian pattern of segregation.

7. Expressed Material

The levels of CP4 EPSPS protein were assessed by a validated enzyme-linked immunosorbent assay (ELISA). Tissues of MON 87705 and conventional soybean control A3525 planted using a randomized complete block field design were collected during the 2007/2008 growing season from five field sites in the Chile.

The mean CP4 EPSPS protein levels of MON 87705 that are relevant to the food/feed safety assessment across the five sites in different tissue types were highest in leaf (ranging from 200 µg/g dw to 530 µg/g dw), followed by forage (120 µg/g dw), seed (110 µg/g dw) and root (77 µg/g dw). As expected, the levels of the CP4 EPSPS protein from the conventional control, A3525, were less than the assay limits of detection (LOD) or limit of quantitation (LOQ) across all tissue types (Monsanto, Part VI, Section 3. pp. 103-104; Geng and Niemeyer, 2009).

8. Toxicological Assessment

The results of the SDS PAGE and western blot assays demonstrate that CP4 EPSPS protein is rapidly degraded in simulated gastric fluid containing pepsin within 15 seconds (Leach et al., 2002). Specific activity of CP4 EPSPS was also significantly diminished by 95% within 15 minutes. The estimated T50 result is <15 seconds.

The effect of heat treatment on CP4 EPSPS protein was evaluated using a functional assay to assess the impact of temperature on activity and using SDS-PAGE to assess the impact of temperature on protein integrity. Results of this study demonstrate that the CP4 EPSPS protein is functionally active at 25 °C and 37 °C. At 55 °C, a decrease in functional activity was observed, specifically the functional activity decreased to 70% of the control when treated for 15 min and to 25 % of control activity when treated for 30 min. The amount of CP4 EPSPS activity remaining following heat treatment for both 15 and 30 min at 75 °C and 95 °C was below the limit of detection. SDS-PAGE analysis showed no significant change in band intensity of the CP4 EPSPS protein due heat treatment. The estimated T50 result for heat inactivation is above 55 °C.

Bioinformatics analyses using FASTA sequence alignment program and TOX_2009 protein database provided by the developer indicated that CP4 EPSPS has no significant homology to any known toxin (Tu and Silvanovich, 2010).

Acute oral toxicity study indicated no treatment related adverse effects on survival, clinical observations, body weight gain, food consumption or gross pathology of mice administered with CP4 EPSPS protein (Naylor, 1993). The NOEL for CP4 EPSPS is 572 mg/kg bw (Naylor, 1993).

9. Allergenicity Assessment

CP4 EPSPS is indeed digestible using SGF with pepsin as the main digesting enzyme. The SDSPAGE followed by Western blot analysis also clearly demonstrated that more than 95% of the CP4 EPSPS protein was already degraded at just below 15s.

The SDS-PAGE analysis and functional assay analysis of CP4 EPSPS protein provided by the developer indicated the functional activity was below the LOD of the assay when incubated at 75 ºC or higher for either 15 or 30 minutes. The activity is significantly impacted by heat treatment (Hernan et al., 2011).

FASTA sequence alignment program and 8-amino acid window search (ALLERGENSEARCH) was used to check if the CP4 EPSPS is homologous to any known allergens, gliadins, and glutenins in FARRP allergen database (AD_2010). Result showed no alignments between CP4 EPSPS protein and sequences with allergenicity concerns. Also, it does not share any eight amino acid matches with proteins in AD_2010 database. This means that CP4 EPSPS does not have any structurally or immunologically relevant similarities with any known allergens, gliadins, and glutenins.

Conventional soybean is already known to contain endogenous allergens and the developer stated that the genetic modifications that transpire did not alter the levels of know allergens that exist in soybean. The experiment conducted also demonstrated that compared to conventional soybean there was no difference in the levels of the allergen present. Therefore, MON 87705 will not pose any more risk than what was original present in conventional soybean.

10. Nutritional Data

Compositional analyses were conducted on seed and forage of MON 87705 and the near isogenic conventional soybean control (A3525) harvested from soybean grown at five sites across Chile in 2007-2008. Commercial reference soybean varieties were included at each site of the field production to provide data on natural variability of each compositional component analyzed. Nutrients assessed in this analysis included proximates (ash, fat, moisture, protein, and carbohydrates by calculation), acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acids (18 components), fatty acids (26, C8-C24), and vitamin E in seed; and proximates, ADF, and NDF in forage. The anti-nutrients assessed in seed included trypsin inhibitors, phytic acid, lectin, isoflavones (daidzein, glycitein, and genistein), raffinose, and stachyose.

Compositional data confirmed that seed and forage from MON 87705 is compositionally equivalent to conventional soybean, except for intended changes in the levels of fatty acids 16:0 palmitic, 18:0 stearic, 18:1 oleic, and 18:2 linoleic.

11. Recommendation

BPI-PPSSD, BAI and STRPs find scientific evidence that the regulated article applied for direct use as food and feed or for processing is as safe as its conventional counterpart and shall not pose greater risk to human and animal health.

DENR Biosafety Committee (Environmental Safety)

After a comprehensive review and evaluation of the documents including the scientific evidence from references and literature submitted by Monsanto Philippines, Inc., on its application for Direct Use as FFP of Soybean MON87705, hereunder are the observations and appropriate actions:

1. The direct use of the regulated article whether for food, feed or for processing will not cause any significant adverse effect on the environment (land and water) and biodiversity. The transgenic crop will not increase its weediness potential in case the seeds spill out into the environment because the CP4 EPSPS protein product produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions such as high temperatures (65°C and above), varying pH, enzyme digestion, etc. (Okunuki et al., 2002);
2. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment and non-target organisms as well as the mitigating measures and contingency plan. Furthermore, the chances of unintended release or planting of the regulated article is very minimal and will not cause any damaging and lasting effects because the receiving environment (areas near the port, roads, railways, etc.) is not conducive for plant growth. Also, soybeans generally are very highly domesticated and do not survive well without human intervention (FAO, 2014).

DOH Biosafety Committee (Environmental Health Safety)

The DOH-BC, after thorough review of the documents, find that the regulated article applied for Direct Use as Food, Feed or for Processing (FFP) is safe as its conventional

counterpart and shall not pose any significant risk to human and animal health and environment.

The following are the observations and recommendations:

1. Scientific pieces of evidence from Toxicity studies and references, find that the regulated article will not cause significant adverse health effects to human and animal health.
2. Dietary exposure to the regulated article is unlikely to result in allergic reaction.
3. The regulated article is as safe as food or feed derived from conventional soybean varieties.
4. The regulated article is not materially different in nutritional composition from that of the non-transgenic soybean or the conventional soybean
5. It is suggested that the Bureau of Plant Industry (BPI) ensure that there shall be clear instructions that the product is only for the purpose of direct use for FFP and is not to be used as planting materials.

SEC Expert (Socio-Economic Considerations)

According to the SEC Expert, local soybean production remains minimal and no significant change is expected through MY 18/19. This is the reason why the Philippines is said to be the largest market for US soybean meal (SBM) given the expanding demand by the hog and poultry industry.

The pattern of production will not be affected since importation is only for food, feed and/or processing and not for production. Given the size of local production, the importation is necessary to cover the unserved demand for soybean meal and other soybean products.

The SEC Expert added that there should be no effect on the cultural practices of farmers since the imported soybean products are not meant for production but rather for direct use as food and feed or for processing.

After a thorough and scientific review and evaluation of the documents provided by Monsanto Philippines Inc., relevant to soybean MON87705, the SEC Expert recommends the approval and issuance of biosafety permit for direct use as food and feed or for processing.